COVID-19 Pharmacotherapy: Drug Development, Repurposing of Drugs, and the Role of the G6PD Enzyme in Determining Pharmacogenomic Outcomes of Potential Drug Candidates

Presented to the S. Daniel Abraham Honors Program

in Partial Fulfillment of the

Requirements for Completion of the Program

Stern College for Women
Yeshiva University
May 2022

Rebecca Bock

Mentor: Dr. Chaya Rapp, Chemistry

Table of Contents

Abstract

The SARS-CoV-2 virus has been the subject of intense pharmacological research. Various pharmacotherapeutic approaches including anti-viral and immunotherapy are being explored. Responding to a pandemic, however, cannot depend on the development of new drugs; the time required for conventional drug discovery and development is far too lengthy. As such, this paper discusses how repurposing drugs is being used as a viable approach for identifying pharmacological agents for treating COVID-19 infections. In evaluating repurposed drug candidates with pharmacogenomic analysis, near-term pharmacological remedies for COVID-19 can be identified. The paper also explores how amplification of the G6PD enzyme gene may be necessary to develop an assay for polymorphisms in this gene which will, thereby, help determine the extent of the therapeutic impact of potential drug candidates in the treatment of COVID-19. If successful, the G6PD gene can be labeled as a crucial factor for consideration in choosing the most effective drug treatment against SARS-CoV-2 for each individual patient.

Key words

Antiviral therapy, immunotherapy, repurposing, pharmacogenomics

I. Introduction

The cause of the COVID-19 pandemic is the highly infectious Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) belonging to the genus of Betacoronavirus which consists of six additional species of the virus [1]. These coronaviruses are spherical in structure, contain single-stranded positive-sense RNA, and are surrounded by protein "spikes" which appear like a crown, giving the virus its name "Corona" [2, 3]. SARS-CoV-2 consists of 29,903 nucleotides with 79-89% sequence similarity to SARS-CoV and 50-60% sequence similarity to MERS-CoV [1,4], two additional forms of coronavirus which have caused previous epidemics [2].

The genome of the virus includes 12 functional open-reading frames (ORFs) which code for both structural and nonstructural proteins which are crucial for virion synthesis and assembly [2]. Some of the structural proteins include the spike (S) protein, the membrane (M) protein, the envelope (E) protein, and the nucleocapsid (N) protein, each of which join to form the outer layers of the virus [2]. Additionally, nonstructural proteins, such as 3-chymotrypsin-like protease (3CLpro), papain-like protease (PLpro), helicase (Hel), RNA-dependent RNA polymerase (RdRp), endoribonuclease, and multiple accessory proteins are encoded for use during viral replication [2,4].

The S proteins are transmembrane glycoproteins which can be found as trimers on the surface of the virion [2]. They can be characterized as a class I fusion protein and are comprised of two subunits, one of which (S1 subunit) allows the virion to attach to receptors on the surface of the host cell and one of which (S2 subunit) causes the membranes of the virion and host cell to fuse,

resulting in entry of the viral RNA into the host cell [2]. The S1 subunit includes one signal peptide, an N-terminal domain (NTD), and a receptor-binding domain (RBD), providing the ability to bind to the host cell receptors as needed. The S2 subunit includes the C-terminal, fusion peptide (FP), heptad repeat (HR) 1 and 2, transmembrane domain (TM), and cytoplasmic domain (CP), providing the ability to fuse with the host cell [2]. These S proteins show approximately a 76% amino acid sequence homology to the S proteins of SARS-CoV, revealing similar viral characteristics, and thus similar activity, between the two viruses [4].

To be successful in receptor-binding, the SARS-CoV-2 S protein must have a clear binding path to the angiotensin converting enzyme 2 (ACE2) receptor of the host cell. Because alveolar epithelial cells on the surface of the lungs and many cells on extrapulmonary tissues are cells with ACE2 receptors, SARS-CoV-2 can easily invade these areas and use them as the starting point for further replication, distribution, and destruction [2]. Due to a mutation in the S protein of SARS-CoV-2, the virus has a greater receptor binding affinity and is, therefore, more transmittable than other coronaviruses [4].

II. COVID-19 Therapeutic Methods

To address the disease, many therapeutic methods have been suggested as means of treating infected patients and ultimately contributing to bringing this worldwide pandemic to a near end. Some strategies involve targeting the virus by either inhibiting viral replication and translation of viral RNA or preventing the virus from binding to host cell receptors, while others involve a focus on the host by either an improvement of the host's immune response or preventing certain host cell enzymes from carrying out their proper function [1]. In each case, many factors must be

considered to determine the most effective strategy, including various characteristics of both the virus and the drugs or other therapies which will be used, such as the composition, functionality, and mechanism of action [2]. By categorizing the numerous types of therapies, the effectiveness of each can be more easily evaluated, helping to find the most efficient way to treat COVID-19 infections [5].

A. Antiviral Drug Therapy

Antiviral therapy aims to inhibit the viral processes that allow the virus to infect the host. Certain drugs do so by targeting functional regions of the virus which are necessary for initial infection by the virus, such as the receptor-binding sites. Others inhibit crucial steps in the replication and translation processes, preventing further or proper synthesis of the virions. By causing changes in the steps which typically provide the virus with its functionality, the virus is unable to infect and replicate [1].

The first method of inhibiting binding, and therefore entry, of the virus into the host cell, aims to prevent initial infection of the host by the virus. To do so, TMPRSS2 (serine protease) activity must be stopped, as it usually cleaves or primes the S protein so that it can bind to the ACE2 receptor. By preventing TMPRSS2 from performing its job, proper binding cannot occur, thereby stopping the virus from infecting its host. One way to do so is with the use of a non-selective TMPRSS2 inhibitor, such as camostat mesilate, or with the use of a selective TMPRSS2 inhibitor, such as bromhexine [2].

An alternative approach to inhibit infection by the virus is by preventing the fusion of the virus with the host cells and the release of viral RNA into the host cells. While the virus requires a specific endosomal pH to fuse with the host cell and for its RNA to enter the cell, some antivirals, including chloroquine, can increase the endosomal pH, thereby stopping the viral RNA from entering the host cell and creating additional copies of the virus [2].

In a case where the viral RNA has already entered the host cell, RdRp inhibitors, such as remdesivir, can be used to interfere with the proper function of the RNA polymerase in the cell [2]. By doing so, the RNA of the virus that has entered the host cell cannot be reverse transcribed properly, or at all, and the virus will not be replicated. Additionally, by inhibiting 3CLpro which is the main protease necessary for replication of the virus, important polyproteins (PP1A and PP1AB) cannot be cleaved to form two independent protein sections which play a role in replication. Therefore, the replication process of the virus would be inhibited by interfering with the proper replication mechanism. This would help to limit the number of overall virions formed and the infectivity of the virus.

B. Immunotherapy

Unlike antiviral therapy, immunotherapy attempts to strengthen the host's humoral immunity to prevent infection more passively, rather than directly targeting the virus itself. However, the two approaches are similar in that, ultimately, the results of the immunotherapy provide antiviral effects. In the immunotherapy process, the antibodies which are part of the host's humoral immune system bind to the antigens of the virus, destroying or blocking the viral cells before they can infect, thereby preventing further infection by the virus [4].

i. Convalescent Plasma

In many cases of infectious diseases, including COVID-19, antibodies formed in individuals who were infected with a pathogen and have recovered are used to fight off infection in other patients [6]. The concept of convalescent plasma suggests providing suffering COVID-19 patients with the plasma, containing IgG antibodies, from previously infected COVID-19 patients. In doing so, the goal is for the transfused antibodies to recognize the virus in the new host and help fight off the infection [4]. As a result, the virus can be neutralized, leading to the reduction of inflammatory response and improvement of symptoms [7].

ii. Monoclonal Antibodies

Monoclonal antibodies (mAbs) are the homogenous products of a single B-cell clone which bind to a specific epitope, i.e., part of the viral antigen [4]. They are effective at stopping viral entry into the host cells by binding to the virus's receptor-binding domain on the S protein, thereby preventing attachment of the virus to the host cell receptors. In addition, mAbs can suppress inflammation caused by increased levels of pro-inflammatory factors as a result of infection by the virus which helps to limit the severity of patients' symptoms. For example, the Interleukin-6 (IL-6) cytokine is greatly involved in cytokine storms, a part of the COVID-19 immune response which can result in extremely high levels of inflammation in infected patients. Monoclonal antibodies, such as tocilizumab and sarilumab, suppress and inhibit IL-6 which helps to prevent an excessive cytokine production and, thereby, improve the outcome of the patient's condition [7].

C. Vaccines

Similar to immunotherapy, vaccines focus more on the formation of antibodies which will be responsible for fighting off the virus. Current and potential vaccines for COVID-19 come in many forms, including live attenuated vaccines, inactivated virus vaccines, recombinant protein vaccines, adenovirus vector vaccines, and nucleic acid (mRNA and DNA) vaccines [6]. They each use different methods, such as distributing portions of the S protein or encoding the viral S protein with viral mRNA or gene fragments, as used in the Moderna mRNA vaccine, to produce neutralizing antibodies and trigger a strong immune response for fighting the viral infection. In all cases, the goal is to create a strong antiviral immune memory which will help to protect the host from infection [7].

Table 1 lists the various therapeutic agents that have either been approved or are in development for use by COVID-19 patients. These drugs consist of a variety of types, including antivirals, anti-parasites, immunotherapies, corticosteroids, monoclonal antibodies, and more. Each drug has a different mechanism of action which allows the drug to prevent or fight viral infection by SARS-CoV-2. By targeting major enzymes or proteins in or on the host cells or the virions, these drugs are able to inhibit crucial processes which allow the virus to infect the host cell. While some of the drugs have been FDA approved and can be used on COVID-19 patients, others are still being tested in clinical trials to ensure the safe and effective use against SARS-CoV-2 infection.

Table 1. Therapeutic Agents for COVID-19

Drug	Type	Mechanism of Action	Stage of Development	Source Reference
Remdesivir	antiviral	inhibits RdRp and viral replication	phase 3 clinical trial	Clinical and Exp. Med. (2021) 21:167–179
Lopinavir/Ritonavir	antiviral	inhibits proteases 3CLpro or PLpro	phase 2 clinical trial	Clinical and Exp. Med. (2021) 21:167–179
Favipiravir	antiviral	inhibits RdRp and viral replication	phase 2/3 clinical trial	Drug Repurposing - Hypothesis, Molecular Aspects and Therapeutic Applications, IntechOpen (2020)
Ribavirin	antiviral	inhibits polymerases, intrferes with RNA capping, causes random mutations, and enhances T cell response		
Chemostat mesylate	antiviral	TMPRSS2 inhibitor	phase 1/2 clinical trial	Int. and Emergency Med. (2020)
Nafamostat mesylate	antiviral	TMPRSS2 inhibitor- inhibits S-mediated membrane fusion		
Ivermectin	antiviral/antiparasitic	reduces viral RNA	FDA approved	Int. J. Mol. Sci. (2020) 21, 5559
Chloroquine/Hydroxychloroquine	antiparasite	inhibitis mitogen-activated protein kinases (MAPK)-interferes with glycosylation of ACE2, viral assembly, and budding, proteolytic processing of the M protein, and changes pH- degrades S protein		
Convalescent plasma	immunotherapy	neutralize the virus by detecting epitopes of the virus		
Intravenous immunoglobin	immunotherapy	interferes with B-cell antigen presentation, immune-modulation, and immune substitution		
Corticosteroids	corticosteroids	activates ACE2 and suppress cytokine storm, thereby playing a protective role in respiratory and digestive systems		
Azithromycin		induces expression of interferon and pro-inflammatory cytokines (IL-6 and IL-8)		
Nitric oxide		inhibits viral RNA and protein synthesis, inhibits viral replication by inhibiting palmitoylation of S-protein which affects binding to ACE2 receptor	phase 2 clinical trial	Brain, Behavior, and Immunity 87 (2020) 59–73
Baricitinib	monoclonal antibodies	JAK antagonist		
Sarilumab	monoclonal antibodies	IL-6 receptor antagonist	phase 2/3 clinical trial	Int. J. Mol. Sci. (2020) 21, 5559
Camrelizumab	monoclonal antibodies	blocks PD-1 signaling to rescue exhausted CD8+T cell-restores CD8+T cell activity during viral infections		
Adalimumab	monoclonal antibodies	targets TNF-α		
Tocilizumab	monoclonal antibodies	IL-6 receptor antagonist, inhibits inducement of inflammatory storm	FDA approved for emergency use	Expert Review of Clin. Pharm. (2020) 13:9, 957-975; FACT SHEET FOR HEALTHCARE PROVIDERS: EMERGENCY USE AUTHORIZATION FOR ACTEMRA® (tocilizumab) (2021) [22]

III. Repurposing Drugs for COVID-19

With the escalation of COVID-19, the virus was rampant, spreading rapidly from region to region. The seriousness and severity of the disease did not go unnoticed, and it was soon categorized as a pandemic. Scientists knew that for there to be any hope for survival for many who would become infected, a quick and effective solution would be necessary. Therefore, they turned to the concept of repurposing already existent drugs, i.e., applying developed drugs for

certain indications to treat other illnesses and diseases, as a more efficient way to treat the highly contagious and potentially life-threatening coronavirus [8].

Generally, drug discovery is very time consuming, costly, and a process which can last anywhere from 10-16 years [9]. Therefore, repurposing drugs is more efficient in that it can shorten drug discovery by between 5-7 years, while also significantly decreasing the total cost of development and production, by using drugs that have already been studied. Because repurposing drugs consists of finding new uses for already existing drugs, whether those drugs are old, failed, being investigated, FDA approved, currently on the market, or being used for more recent diseases, the familiarity with the drugs being tested allows for a reduced failure risk, as any toxicity or general safety concerns have already been identified and addressed [9]. By understanding the drugs' level of effectiveness for other diseases that have similar characteristics and symptoms to COVID-19, based on the understanding of the mechanisms of action, the adverse effects, and previous indications, scientists have been able to identify candidate repurposed drugs to treat the virus. Through this process, the scientific community can potentially help bring an end to the pandemic [8].

Originally, repurposed drugs were a serendipitous phenomenon which served great and unexpected benefits to the world of medicine [8]. With the increase in advanced technology and scientific development, repurposing drugs has become a complex but viable process, allowing for accelerated discoveries [9]. Repurposing drugs consists of four stages, including compound identification, compound acquisition, development, and FDA post-market safety monitoring. Because information about the drugs being used for repurposing is already disclosed, including

information on their previous clinical efficacy and level of safety, the process does not require initial years of drug development. This allows for direct entry of the drugs into preclinical testing. Therefore, repurposed drugs can be quickly prepared for efficacy testing so that they can be used to treat rapidly and re-emerging infectious diseases, such as COVID-19, and difficult to treat or neglected diseases.

Repurposed drugs can work as either on-target or off-target drugs [8]. When on-target, the mechanism of action of the particular drug and, therefore, the biological target remain the same. However, the resulting therapeutic outcome can differ from a previous outcome, allowing the drug to be used for a different disease [9]. Contrastingly, off-target drugs do not have the same mechanism of action and, therefore, do not have the same biological target. Consequently, these drugs provide new therapeutic indications which were not previously discovered [9]. To develop these drugs and test for their efficacy, the drugs are first screened *in silico* and then are investigated in vitro, or in vivo, in desired biological targets. Once successful with regards to both stages, the drugs are further evaluated in clinical trials in the hopes of being a safe and effective drug for the disease that requires treating.

Table 2 lists several drugs which have the potential to be repurposed for treatment of COVID-19. Each listed drug has been previously and/or is currently being used to treat other viruses and diseases, including influenza, malaria, rheumatoid arthritis, HIV, and more. If successful, these candidate drugs may have the ability to treat COVID-19 patients, ranging from those who have more mild symptoms to those who are critically ill and hospitalized. Additionally, some of the listed drugs might be more effective when used with other drugs rather than when used alone. By

comparing the treatment status and effectiveness of each drug, scientists can determine which drugs are most advantageous for use against COVID-19.

Table 2. Candidate Repurposed Drugs for COVID-19

Drug	Original Use	Treatment/Usage of Drug	Empirical Studies
Favipiravir	influenza		
Hydroxychloroquine	malaria, rheumatoid arthritis, systemic lupus erythematosus	for treatment of patients in clinical trial and hospitalized patients when necessary	increased efficacy when used with azithromycin
Chloroquine	malaria, extraintestinal	for treatment of patients in clinical trial	less effective than hydroxychloroquine
Ivermectin	HIV, dengue, influenza, RSV, rabies		
Lopinavir/Ritonavir	HIV/AIDS		can be effective together with ribavirin
Remdesivir	influenza, ebola	for patients with severe disease	
Tocilizumab, IL-6 inhibitor	rheumatoid arthritis		
Colchicine	gout, pericarditis, HIV, hepatitis, zika		
Baricitinib and Ruxolitinib	rheumatoid arthritis		

IV. Pharmacogenomics

With the need for a fast and efficient system for repurposing drugs to help treat COVID-19, all possible limitations must be considered. One of these includes the relevance of the human genome in instructing the body's interactions with the different therapeutic drugs that may be used to fight off the virus [10]. In fact, knowing the relationship between variations in expression of certain crucial human genes and different therapeutic outcomes of various drugs, allows for a quicker, safer, and more successful use of repurposed drugs in combatting COVID-19. This concept, employing pharmacogenomics, can lead to a handful of emergency treatment methods, helping to suppress the virus before it has the chance to do further harm [10].

Pharmacogenomics is based on monogenic variants, i.e., genetic polymorphisms, and how these changes affect drug response and efficacy. Genetic variation may result from a single nucleotide polymorphism (SNP) which is the most common of changes. These changes in the genetic material can alter the way in which drugs are recognized, transported, and metabolized within the body which can potentially have harmful results for specific recipients based on the way in which their genetic material is expressed. While some drugs will be more quickly recognized and metabolized by the host's enzymes, others might not be as benign, and may even go as far as to cause adverse effects [11]. Additionally, certain drugs might only need to be administered in small doses while others may be more effective in the presence of another drug. In any case, knowing how a patient's body will react to the intake of specific drugs can help to determine the most favorable and effective doses of such drugs for each person and can help prepare for any potential adverse effects [11], thereby allowing for design of the optimal personalized pharmacotherapy [12].

While variations in the genome of the genes that encode enzymes which metabolize drugs play an extremely significant role in treating COVID-19, changes in the genome of enzymes which are directly related to infection by SARS-CoV-2 can determine how severely one will be infected with the virus [13]. Certain enzymes are more relevant than others with regards to infection by SARS-CoV-2 and, therefore, the genetic makeup of the genes that express those enzymes should be closely studied. For example, ACE2 which controls the magnitude of virus-host cell binding and, thus infection by the virus, is a very important enzyme in the process of viral entry into the host cell. A polymorphism in this enzyme can either help to prevent infection or can result in the promotion of infection based on the change that was made in the amino acids which are

expressed. The same is true of TMPRSS2 which primes the S protein on the surface of the virus, thereby permitting the virus to bind to the host cell's ACE2 receptor and allow for entry of the viral RNA into the host cell. Variations in the genotype of the gene which expresses TMPRSS2 can limit the level of expression or effect of the enzyme's ability to prime the S protein; in effect limiting or preventing binding of the virus to the host cell and entry of viral RNA into the host cell [13]. Therefore, it is important to assess all factors that might impact the level of infection, including variations in enzymes that are important for drug uptake and also enzymes which directly impact viral infection.

When determining the optimal drugs to use for COVID-19 patients, it is extremely important to consider the patients' backgrounds. The world consists of many different populations, with people in each having varied genetic makeup from people of other populations. This is relevant because drastic differences in genetic makeup can result in varied responses to certain drugs [11]. Therefore, categorizing genetic material based on different populations can help scientists get a better sense of how and to what extent crucial enzymes will be expressed in specific populations so that certain drugs can be used specifically for patients of that population. By focusing on the patients' backgrounds and determining their origins and respective population, specific drugs can be used to most effectively target infection by SARS-CoV-2 [10]. Because people of different origins would react differently to varying drugs due to variations in their genetic makeup, it is important to also recognize potential adverse effects which can help in choosing the most effective and safest drugs for each population [11, 12]. This can help to limit the use by patients of ineffective drugs or drugs that have the potential to cause adverse effects for them specifically.

Table 3 lists multiple drugs which are potential repurposed therapeutic agents for COVID-19, in addition to their pharmacogenomic indications. Knowing the genotypes of each drug allows for recognition of any polymorphisms within the genetic material which may result in different forms of expression of critical metabolic enzymes. Each type of alteration in the gene sequence of these important enzymes in the host cells can have a great impact on the effectiveness of the drug. While these polymorphisms can have a negative impact, including lowering enzyme activity or causing adverse effects upon metabolization of the drug, they can also result in greater drug concentrations. Associating specific genotypes with the outcome when using certain drugs allows for determining which drugs are most effective for each infected individual.

Table 3. Pharmacogenomic Indications of COVID-19 Candidate Drugs

Drug	Genotype	Phenotype
Hydroxychloroquine	CYP2D6, CYP3A4, CYP3A5, CYP2C8, CYP1A1 substrate	polymorphism in CYP2C8*4, CYP2C8*2, CYP2C8*3 cause lower enzyme activity, poor or intermediate CYP2D6 metabolizers (CYP2D6*4, CYP2D6*10) result in high hydroxychloroquine concetrations, SNPs in G6PD cause lower enzyme activity result in greater risk of haemolysis
Chloroquine	CYP2D6, CYP3A4, CYP2C8 CYP1A1 substrate	polymorphism in CYP2C8*4, CYP2C8*2, CYP2C8*3 cause lower enzyme activity, SNPs in G6PD cause lower enzyme activity result in greater risk of haemolysis
Lopinavir/Ritonaivr	CYP3A4 inhibitor and substrate, CYP2D6 substrate, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 inducer, Pgp substrate, UGT1A1 inducer	polymorphisms in UGT1A1, UGT1A7, APOE, APOC3 cause gastrointestinal adverse effects, hepatotoxicity, pancreatitis, and cardiac conduction abnormalities.
Ribavirin	CYP3A4 inhibitor and substrate, CYP2D6 substrate, CYP1A2, CYP2C9	polymorphisms in ITPA, VDR, SLC28A2 result in toxicity and adverse effects, variants of VDR, SLC29A1, IFNL3, MICB-OASL result in increased response
Azithromycin	CYP2C9 inhibitor, biliar excretion	two polymorphisms in ABCB1 result in lower peak drug concentrations
Remdesivir	CYP2D6, CYP3A4, CYP2C8, CYP27B inducer, OATP1B1	unknown
Favipiravir	CYP2C8 and aldehyde oxidase inhibitor	unknown
Dexamethasone	CYP3A4, CYP2C8 inducer	variants of CYP3A7, CYP3A5, and CYP3A4 can affect corticosteroid response, variants of ATF5, MIR3683, CTNNB1, PNPLA3

V. G6PD

Pharmacogenomics can be considered an extremely efficient technique when repurposing therapeutic drugs. It allows for the understanding of how treatment patterns with regards to a specific drug, in different populations, can provide insight into the use of those drugs for other

illnesses and within other populations [10]. As COVID-19 continues to rapidly spread and mutate, and the world is desperate for a cure, drugs such as chloroquine or hydroxychloroquine, which are antiparasitic and autoimmune drugs commonly known to treat malaria and lupus, are being thoroughly studied. Because the main source of treatment of these drugs is for malaria and lupus patients, there is a focus on populations most affected by malaria and lupus in order to determine when and in what conditions these drugs are most effective [12]. In fact, the differences in effectiveness can actually be tied to variations within genes of enzymes which are crucial for metabolizing both chloroquine and hydroxychloroquine. As different populations have varying genomes, the populations have variations in existing genes for important enzymes which are critical for major bodily functions [14].

For many decades, chloroquine and hydroxychloroquine have been a key component for treatment and prevenation of malaria. Malaria is a disease caused by the parasitic *Plasmodium* species which invades the host's red blood cells (RBCs) and decomposes available hemoglobin as a means of synthesizing proteins which the parasite needs to grow [15]. In treating malaria, chloroquine and hydroxychloroquine, which are 4-aminoquinoline drugs, inhibit the parasitic heme polymerase, thereby resulting in toxic heme buildup and the prevention of the parasite to synthesize the necessary proteins for further growth [12]. In addition to inhibiting the spread of malaria causing parasites, chloroquine and hydroxychloroquine are used to treat various autoimmune, viral infectious, and rheumatological diseases. When used as an antiviral, the drugs raise the endosomal pH which prevents viral fusion and entry and inhibits the replication and glycosylation of viral proteins, thereby preventing viral assembly and spread [12].

When focusing on the impact of chloroquine and hydroxychloroquine use in regions prevalent with malaria, particularly Africa, researchers noticed a pattern in the severity of malaria cases [16]. They were eager to find a reason for such patterns and an explanation for what might be relevant in understanding the difference in severity among different patients. The necessary step was to investigate the genome of the patients to see if anything gene-related was causing the discrepencies. In doing so, the glucose-6-phosphate dehydrogenase (G6PD) enzyme gene was noticed as playing a role in the severity of malaria in an individual due to the high percentage of G6PD deficient individuals in such populations [16]. In fact, this high G6PD deficient prevalence suggested that being deficient in the enzyme might actually have potential therapeutic and protective effects against malaria [16]. This is significant in that being G6PD deficient can result in haemolytic anemia which is a risk that must be considered when administering aminoquinoline drugs to treat malaria [16]. The different G6PD genetic variants each provides different levels of protection against malaria and each has a greater effectiveness towards a specific species of malaria [16]. Additionally, the genotypic variation determines the extent of phenotypic deficiency which is also relevant to determining the level of protection provided by the G6PD variant [16]. In most cases, hemizygous males and heterozygote females were shown to have the highest level of protection against malaria in populations infected with malaria [16].

As it is clear that the G6PD enzyme plays a very signicant role in determining the use of chloroquine and hydroxychloroquine to treat malaria, studies suggest that G6PD may also be critical in determining treatment by chloroquine and hydroxychloroquine which are drugs that have been presented as potential antiviral therapeutics to be used to treat COVID-19. In identifying a polymorphic region which will be used to determine which repurposed COVID-19

therapeutic drugs will or will not be metabolized by specific populations or by specific phenotypes, three main factors must be considered including the virus, the potential drug, and the enzyme. In the case of SARS-CoV-2, the polymorphic region of the enzyme glucose-6-phosphate dehydrogenase (G6PD) is being studied in order to determine which drugs will be most effective in treating infection by SARS-CoV-2 in the presence of polymorphic variations in the polymorphic region of this enzyme.

Figures 1 and 2 depict two different polymorphic variations in the G6PD enzyme gene, one identified as the G6PD Mediterranean variant and the other as the G6PD A-Haplotype variant. The difference between the two variants is represented by the difference in the single nucleotide polymorphism, with the change in the Mediterranean variant being from a C nucleotide to a T nucleotide at position 154534419, whereas the change for the A-Haplotype variant is from a G nucleotide to an A nucleotide at position 154536002. While both figures represent a polymorphic region of the G6PD enzyme gene, each variant has its own unique polymorphic variation, resulting in different therapeutic outcomes depending on the specific drugs used with each specific variation.

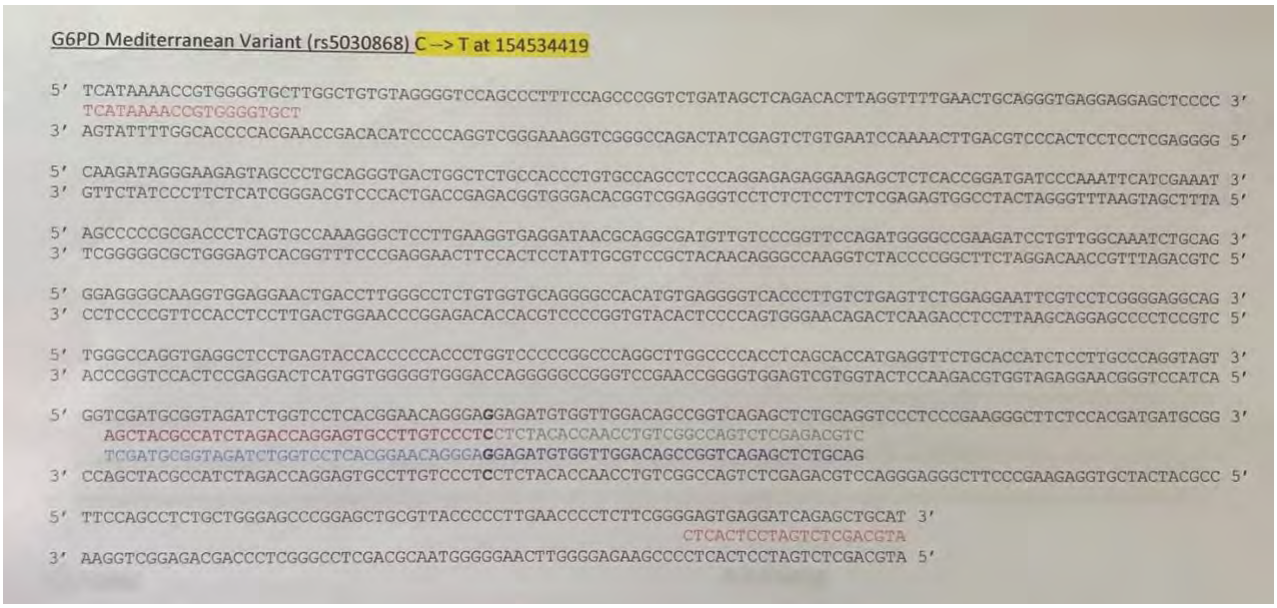

Figure 1: G6PD Mediterranean variant represented by a single nucleotide polymorphism in the polymorphic region of the G6PD enzyme gene.

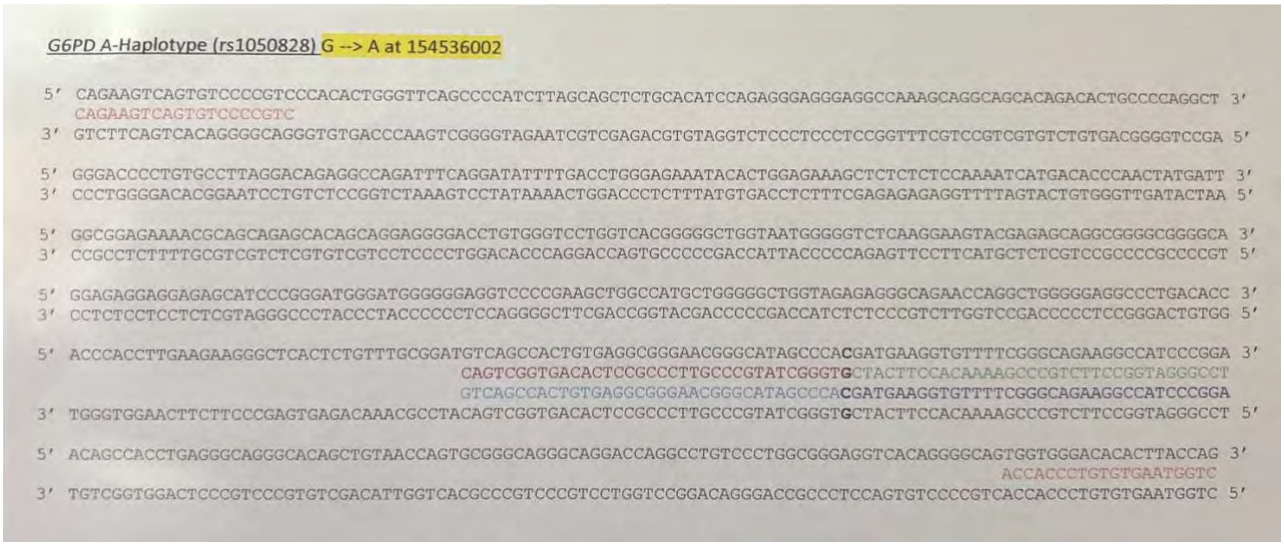

Figure 2: G6PD A-Haplotype variant represented by a single nucleotide polymorphism in the polymorphic region of the G6PD enzyme gene.

To determine whether or not this enzyme plays a significant role in infection by SARS-CoV-2, the first step is to study its genome and its significant role within the body. G6PD is an enzyme which is a key player in the production of nicotinamide adenine dinucleotide phosphate (NADPH), a reducing factor needed for a cellular antioxidant response [14]. In fact, it is the only source of NADPH in red blood cells [16]. In order to produce NADPH, a rate limiting step, known as the pentose phosphate pathway, must take place in which G6PD is the first enzyme. G6PD is also considered to be a housekeeping gene, indicating its involvement in cell maintenance and its expression in many different cells in many different species [14]. Because red blood cells are not nucleated and, therefore, are unable to replenish the G6PD that has been degraded, the older the blood cells get, the more deficient they are in active G6PD [16]. These blood cells will then be more likely to undergo oxidant haemolysis. However, this is not an uncommon phenomenon, as G6PD deficiency is said to be the most common human enzyme abnormality [16].

Based on its widespread presence, G6PD is seen to have numerous amounts of polymorphic variations, with most differences only indicated by a single base change. With these polymorphic variations come various levels of enzymatic activity, with some cells having a high degree and some having a decreased rate, thereby affecting the enzyme's ability to protect the cell from oxidative challenges such as oxidant haemolysis [14]. In fact, G6PD deficiency variants can be categorized into four different groups of variants: Class I, II, III, and IV, in decreasing order of severity. Class I variants represent the most severe form of G6PD deficiency which results in chronic non-spherocytic hemolytic anemia. Class II variants, like Class I variants, have less than 10% enzyme activity as compared to a normal enzyme activity level. However, Class II variants

do not cause hemolytic anemia as do Class I variants. Lastly, Class III and IV variants are more mild and only cause hemolysis in cases of extreme levels of oxidative stress [17].

When the body is infected by an unknown organism, more specifically a virus such as SARS-CoV-2, the immune system will respond by generating antioxidants in order to overcome the oxidative stress caused by infection. In such a case, inflammation will occur due to both infection of the virus and as a result of the immune response. The reduced antioxidant, glutathione (GSH), is responsible for controlling reactive oxygen species (ROS) production by eliminating unstable radicals which cause oxidative stress, a process which is curcial for modulating such oxidative stress. G6PD is significant in the generation of reduced glutathione in that G6PD catalyzes the production of NADPH. This is important because the NADPH is a co-factor for a different enzyme, glutathione reductase, which is responsible for the reduction of glutathione disulfide into reduced glutathione. Because G6PD plays a pivitol role in maintaining redox homeostasis and in the immune response to infection of a host, a deficiency in the enzyme would limit the cells' ability to respond efficiently to oxidative stress caused by the virus. With less G6PD, not enough GSH will be generated which means the level of uncontrolled ROS production will be higher and, therefore, the level of oxidative protection will not be as high. This G6PD deficiency can be very important to consider when searching for a therapeutic drug that can help treat infection by SARS-CoV-2 because any drug that might provide oxidative triggers will only cause more harm for a patient who is already unable to generate enough GSH to overcome the oxidative challenges caused by infection. Additionally, in a case where viral infection causes high levels of oxidative stress, a host with G6PD deficiency will be increasingly

unable to sufficiently combat infection by the virus, thus resulting in more severe symptoms of infection [17].

In order to determine whether there is even a reason to be concerned about severe symptoms due to increased oxidative stress, it is important to consider the host's genome with regards to the G6PD enzyme. G6PD deficiency is an X-linked enzyme deficiency that can affect both males and females, although it primarily affects males. As males only have one X chromosome, they can either be phenotypically normal or deficient depending on whether or not there is a polymorphic variation in the G6PD gene. If the male is a hemizygote, he will be phenotypically G6PD deficient. For females, because they have two X chromosomes, a heterozygote has the ability to have a range of G6PD deficiency due to random inactivation of the X chromosome. Therefore, heterozygous females with the deficiency will not necessarily have as extreme of a case of deficiency as hemizygous males with the deficiency. In fact, the homozygous females will be at the same level of deficiency as the hemizygous males. For homozygous females or hemizygous males who both carry the highest level of G6PD deficiency, further problems can arise upon infection by a virus due to the largely decreased levels of G6PD enzymatic activity. The G6PD deficiency is found to be most highly prevelant in the Meditteranean, Asia, and Africa and would, therefore, have a higher rate of causing such complications in those areas [17].

VI. Design Considerations for a Pharmacogenomic Assay

In determining the role of the G6PD enzyme in the metabolization of therapeutic candidates for treating COVID-19, a pharmacological assay must be designed and performed. An important point to consider is that pharmacogenomics, the intersection of pharmacology and genomics,

entails analysis of a specific segment of genomic DNA that contains polymorphic region(s). Common polymorphisms or variants include single nucleotide polymorphisms (SNP), insertions or deletions, and copy number variants [18]. Luckily, this is true regarding the G6PD enzyme which serves as a perfect candidate for investigation.

Numerous genotyping technologies are available to determine an individual's genotype. A technology platform is typically chosen based on access to instrumentation, technical capabilities, throughput requirements, the nature of the polymorphism, the speed at which the genotyping must be performed, and the cost [19]. For clinical pharmacogenomic (PGx) applications, the ideal platform should reliably and accurately detect an individual molecular change (variants) occurring in each selected gene associated with drug response (Pharmacogene). In addition, the gene(s)-drug pair needs to be curated at the allele level to properly confer proven clinical validity and utility. There are regulatory organizations like the Federal Drug and Food Administration (FDA) and professional organizations such as the Clinical Pharmacogenomics International Consortium (CPIC) that produce standards for the proper and safe implementation of pharmacogenomic testing in clinical practice. Any test that is used for patient pharmacotherapy decisions must be performed in a certified clinical laboratory. In the United States, the certification must be compliant with Clinical Laboratory Improvement Amendments (CLIA) to ensure that the clinical laboratory operates under standards for the overall quality management system to ensure best clinical laboratory practices.

The most common methodological platforms used in the field of PGx are Next-Generation Sequencing (NGS), the polymerase chain reaction (PCR), and microarrays. Each approach offers

a unique combination of scale, accuracy, throughput, cost, and has distinct technical and operational advantages and dis-advantages [20].

In order to determine the proper segment of genomic DNA on which to focus as a way of identifying which drugs will be most effective in treating COVID-19 in each patient, ligase chain reaction (LCR) can be used along with polymerase chain reaction (PCR). LCR is a DNA amplification method which allows for detection of nucleic acid polymorphisms related to genetic disorders or pathogenic infection. LCR provides the ability to accurately target a single base pair change through its use of a thermostable ligase and its ability to be used with a primary amplification method. This method is typically used in conjunction with polymerase chain reaction (PCR), another very popular form of DNA amplification. In using LCR, two adjacent synthetic oligonucleotide primers ligate through the hybridization to a single strand of the target DNA. Additionally, there is a second pair of primers which is complementary to the first and which has a nucleotide at the 3' end of the upstream primer representing the change in the DNA. In order for the ligation of the two adjacent synthetic oligonucleotide primers to occur, the 3' end of the upstream primer will primarily line up with the targeted sequence at a position where there is a detected single base pair change. If the 3' end of the upstream primer and the position with the detected single base pair change are complementary, the two adjacent synthetic oligonucleotide primers can ligate. These ligated primers can then act as templates for further hybridization and ligation which will allow for exponential amplification, similar to PCR. In a case where the primers don't match the detected single base pair change, there will be no ligation of the primers which will indicate that there truly is a base pair change in the target sequence [21].

Through the use of thermostable ligase, which allows for ligation within a range of temperatures close to the melting temperature (T_m), and through the use of LCR primers which have a single base pair overhang, as opposed to blunt ends, target-independent ligation can be reduced. Additionally, in order to avoid target-independent ligation, the primers cannot be designed in a way which allows them to act as a template for the ligation of other primers. Therefore, to prevent ligation of the 3' ends of the primers, the nonadjacent 5' ends should have tails of noncomplementary base pairs. Depending on the specific single base pair change in the DNA, different primers with different 3' ends will produce either more or less efficient ligation. Primers with G and C complementary nucleotides at the 3' end are more likely to provide more efficient ligation than A and T complementary nucleotides due to the greater hybridization as a result of the greater amount of hydrogen bonding between G and C nucleotides [21].

VII. Conclusion

The world has been struck by an unprecedented crisis with the emergence of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-Cov-2) and its rapid spread and escalation to the COVID-19 pandemic. While there is a clear need for new drugs to combat the deadly virus, research and development of new medications can take many years. Looking to existing drugs that can be repurposed to treat patients with COVID-19 will be critical to mounting a timely and effective pandemic response and to saving lives. Pharmacogenomics, an emerging field at the intersection of genetics and pharmacotherapy, may be a key player in enhancing the success of repurposing existing drugs to alleviate the urgent global situation. To assist in this task, the G6PD enzyme gene may be relevant to determining whether or not certain drugs can be used to

treat COVID-19 in varying populations without causing adverse effects to the individuals of those populations. While the COVID-19 pandemic did not allow for thorough in person research on the significance of the G6PD enzyme with regards to therapeutic treatments of COVID-19, further experimentation will be conducted to hopefully provide the necessary data in making such a determination.

Acknowledgements

Dr. Wachtell

Thank you for all the effort you put into making the Honors Program one which is filled with numerous interesting events and great opportunities. Having the ability to share such unique experiences with peers and friends has really opened my eyes and led me to realize how fortunate I am to have been part of such a special program.

Dr. Rapp

Thank you, Dr. Rapp, for all you have taught me in my years in Stern. Starting my first year, in Chemistry, I remember sitting every day in class enwrapped in your words. From that point forward, I knew you would be someone I could trust to and would be eager to have guide me through my choice to become a pharmacist and in completing my honors thesis. I feel so privileged to have had the opportunity to be your student and now, someone you mentor. Just knowing all your accomplishments is truly inspiring but having the opportunity to work so closely with you is something which has taught me an innumerable amount.

Dr. Loewy

A massive thank you to you, Dr. Loewy, for being the reason I have a thesis at all. My gratitude and appreciation for all of your help, guidance, mentorship, and

kindness are more than I can express. You so willingly provided me with the opportunity to conduct research with you last summer and over this past semester, giving me the tools and support necessary to publish a scientific research paper on which my thesis is based. Not only have you provided me this opportunity, but you have been there to guide me every step of the way, encouraging me in my path to be a pharmacist, advising me in all decisions, and assisting me with whatever I needed. Working with you has been a great privilege, and I look forward to continuing to work with you in Touro College of Pharmacy over the next four years.

My Parents

Thank you for everything you do for me, each and every day. I truly could not have made it to this point without the support, love, and encouragement you have given me. For as long as I can remember, you have constantly pushed me to be the best student and person I can be, giving me the confidence I needed to excel in my studies, the values to create the most meaningful friendships and relationships, and the motivation to put my all into everything I set my heart out to do. Thank you for always believing in me and for being the greatest role models. Love you tons!

My Siblings

Thank you for being my best friends and people I know I could always count on. I feel so lucky to have each one of you as such a big part of my life. You guys have always been the ones I look up to and call when in need for life advice. Thank you for filling my life with the best memories and moments I will never forget. Love you guys beyond!

My Fiancé

To my dearest Daniel, thank you for the smiles you put on my face every single day and thank you for always cheering me on and telling me I could do it, even in the times when I'm confident I can't. You have been the biggest highlight to my last year in Stern and I can't wait for what else is to come. I am so lucky and thankful to have you and love you so much!

My Friends

Thank you all for changing my life and being the main reason why I've made it through college. Thank you for always being my source of entertainment and happiness and, when necessary, my shoulder to cry on. You all mean the world to me, and I could not have asked for more incredible friends. Love you guys!

REFERENCES

1. Rohani N, Moughari F.A, Eslahchi C. DisCoVering potential candidates of RNAi-based therapy for COVID-19 using computational methods. PeerJ. 2021.
2. Depfenhart M, de Villiers D, Lemperle G, et al. Potential new treatment strategies for COVID-19: is there a role for bromhexine as add-on therapy? Int. and Emergency Med. 2020.
3. Loewy Z.G, The journey to a COVID-19 diagnostic test. Journal of Precision Medicine. June 2020.
4. Zhand S, Jazi M.S, Mohammadi S, et al. COVID-19: The Immune Responses and Clinial Therapy Candidates. Int. J. of Molec. Sci. 2020, 21, 5559.
5. Gavriatopoulou M, Ntanasis-Stathopoulos I, Korompoki E, et al. Emerging treatment strategies for COVID-19 infection. Clinical and Exp. Med. 2021, 21:167–179.
6. Yang X, Liu Y, Yang Q, et al. Medication therapy strategies for the coronavirus disease 2019 (COVID-19): recent progress and challenges. Expert Review of Clin. Pharm. 2020, 13:9, 957-975.
7. Zhang J, Xie B, Hashimoto K. Current status of potential therapeutic candidates for the COVID-19 crisis. Brain, Behav., and, Imm. 2020, 87:59-73.
8. Parvathaneni V, Gupta V. Utilizing drug repurposing against COVID-19 – Efficacy, limitations, and T challenges. Life Sciences. 2020, 259.
9. Rudrapal M, Khairnar S.J, Jadhav A.G. Drug Repurposing (DR): An Emerging Approach in Drug Discovery. IntechOpen. 2020.
10. Badary, O.A. Pharmacogenomics and COVID-19: clinical implications of human genome interactions with repurposed drugs. The Pharm. J. 2021, 21:275-284.

11. Cafiero, C. Re, A. Micera, A, et al. Pharmacogenomics and Pharmacogenetics: In Silico Prediction of Drug Effects in Treatments for Novel Coronavirus SARS-CoV2 Disease. Pharm. And Personalized Med. 2020, 13:463-484.
12. Babayeva M, Loewy, Z. Repurposing Drugs for COVID-19: Pharmacokinetics and Pharmacogenomics of Chloroquine and Hydroxychloroquine. Pharmacogenomics and Personalized Medicine 2020, 13: 531–542.
13. Al-Eitan, L.N, Alahmad, S.Z. Pharmacogenomics of genetic polymorphism within the genes responsible for SARS_CoV-2 susceptibility and the drug-metabolising genes used in treatment. Rev. Med. Virol. 2021, 31:294.
14. Bancone G, Chu C.S. G6PD Variants and Haemolytic Sensitivity to Primaquine and Other Drugs. Frontiers in Pharmocology 2021, 12:638885.
15. Lei ZN, Wu ZX, Dong S, et al. Chloroquine and hydroxychloroquine in the treatment of malaria and repurposing in treating COVID-19. Pharmacol. Ther. 2020, 216: 107672.
16. Awab G.R., Aaram F, Jamornthanyawat N, et al. Protective effect of Mediterranean-type glucose-6-phosphate dehydrogenase deficiency against *Plasmodium vivax* malaria. Elife 2021, 10: e62448.
17. Youssef, J.G, Zahiruddin F, Youssef G, et al. G6PD deficiency and severity of COVID19 pneumonia and acute respiratory distress syndrome: tip of the iceberg? Ann Hematol. 2021, 100(3): 667-673.
18. Liu, J., Zhou, Y., Liu, S., Song, X., Yang, X., Fan, Y., Chen, W., Akedemir, Z.C., Yan, Z., Zuo, Y., Du, R., Liu, Z., Yuan, B., Zhao, S., Liu, G., Chen, Y., Zhao, Y., Lin, M., Zhu, Q., Niu, Y., Liu, P., Ikegawa, S., Song, Y-Q., Posey, J., Qiu, G., Zhang, F, Wu, Z., Lupski, J., and Wu, N. (2018) The coestistence of copy number variations (CNVs) and

single nucleoide polymorphisms (SNPs) at a locus can result in distorted calculations of the significance in associating SNPs to disease. Hum. Genet. 137: 553-567.

19. Offit, K. (2011) Personalized medicine: new genomics, old lessons. Hum. Genet. 130: 3–14.
20. Zhang, J., Chiodini, R., Badr, A. and Zhang, G. (2011) The impact of next-generation sequencing on genomics. J. Genet. Genomics 38: 95-109.
21. Wiedmann M, Wilson W.J., Czajka J, et al. Ligase Chain Reaction (LCR) – Overview and Applications. Genome Research 1994, 3: S51-S64.
22. Fact Sheet for Healthcare Providers: Emergency Use Authorization (EUA) for ACTEMRA® (tocilizumab). South San Francisco, CA: Genentech, Inc; June 24, 2021.